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CONTEMPORARY DATA ON MEASURES TAKEN IN THE USSR
AGAINST BRUCELLOSIS OF LIVESTOCK

Prof S. N. Vyshelesskiy
 Active Member, Acad Sci Belorussian SSR

This report does not deal with the entire complex problem of eradicating brucellosis, a problem which has not been solved in any country. We only want to deal with those special methods of eliminating brucellosis which have been developed by Soviet specialists.

To liquidate brucellosis, it is necessary, above all, to find the source of the disease, i.e., to recognize its carriers and transmitters and to render them harmless. At present, science disposes of a number of serological and allergical diagnostic methods for brucellosis. Their combined use permits, if not immediate, at least gradual recognition of the dangerous transmitters of brucellosis in the infected herd and their isolation or removal to the slaughterhouse for utilization as meat.

Until recently, the official instructions of the Ministry of Agriculture USSR prescribed as diagnostic methods the agglutination reaction (test-tube method) and the allergy reaction with the use of abortine, brucellizate, and brucellohydrolyzate preparations. At present, abortine has gone almost out of use, despite its wide use in combination with the agglutination reactions (1939 - 1940) in four oblasts of the USSR and despite the fact that it gave fairly good results. Only this year was brucellohydrolyzate authorized for practical use by the Veterinary Administration of the Main Administration of Animal Husbandry of the Ministry of Agriculture USSR. In medical practice, the cytophage reaction has found use as a supplementary diagnostic method, but it has not caught on in veterinary practice.

Lately, a ring test for detecting brucellosis has been proposed in the investigation of milk. It was checked by the staff of the Chair of Epizootics of the Moscow Veterinary Academy, by the Brucellosis Laboratory of the All-Union Institute of Experimental Veterinary Medicine, and by several scientific workers of other veterinary laboratories, but up to now has not yet received official approval.

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In accordance with the instructions ineffect, the basis for the diagnosis of brucellosis of cattle were the agglutination and the allergy reactions; the basis for the diagnosis of brucellosis of sheep was the allergy reaction with the aid of brucellizate and, most recently, brucellohydrolyzate. Quite recently, the Veterinary Administration of the Main Administration of Animal Husbandry of the Ministry of Agriculture also recommended the complement fixation reaction as a supplementary diagnosis method.

Scientific and practical evaluation of the above methods was carried out by a number of Soviet veterinary scientists: Lokteva, Novochoerkassk; N. Ye. Tsvetkov, Leningrad; Ye. S. Orlov, M. I. Chernysheva, O. I. Moryakova, and V. M. Krasov, All-Union Institute of Experimental Veterinary Medicine; K. P. Studentsov, Kazan Scientific Research Veterinary Institute; and A. I. Gromyko, P. D. Kovalenko, D. K. Bessonov, Zhovanik, and others.

On the basis of the work of the above authors, and also of recent investigations carried out by Z. N. Kostrulina and P. P. Samoylov, candidates of the Chair of Epizootics of the Moscow Veterinary Academy, the diagnostic significance of the sero-allergic method of detecting brucellosis may be evaluated in the following manner. Neither the agglutination reaction in two variants (test-tube and drop-plate methods), nor the complement fixation reaction, nor the allergy preparations, when used individually, will detect all animals infected with brucellosis, carriers and transmitters of the infection. Only the combined use of all these methods can provide better results in the detection of brucellosis sources, and consequently, also chances for liquidating the infection.

The agglutination reaction (test-tube method) is not perfect, since it does not detect all brucellosis cases. The reaction is sometimes retarded in the initial stage of the infection and fades out soon, while the infection has not yet passed. Sometimes the agglutination reaction disappears temporarily, only to return after a month or sometimes even after longer intervals. In addition, it yields a higher percentage of doubtful indications than the complement fixation reaction. However, it does have an advantage over the other methods. It generally appears in diseased animals earlier than the other reactions and allows quicker detection and isolation of animals afflicted with brucellosis, thereby preventing spread of the infection.

The test tube method of carrying out the agglutination reaction, generally used on cattle, is not very feasible for use on sheep. According to the investigations of Z. N. Kostrulina, the test-tube and the drop-plate methods supplied positive or doubtful results within 15 to 20 days after the experimental infection of sheep, but the test-tube reaction faded 109 days after infection, while the drop-plate method supplied positive results for a longer time and disappeared only after 220 days.

According to the data of O. I. Moryakova and Il'in, the blood-drop method supplies indications analogous to the drop method with serum, but it has not yet been completely worked out and has not yet been approved. The drop-plate agglutination method is completely feasible for diagnosis of brucellosis also in sheep, and has been developed to a sufficiently high degree, in particular by Z. N. Kostrulina and P. P. Samoylov.

The complement fixation reaction, as shown by many Soviet veterinary researchers, has considerable advantages over the agglutination reaction both in the case of cattle and sheep, giving a high percentage of positive reactions, and is exhibited by the living organism of the animal suffering from brucellosis several months longer than the agglutination reaction. But its wide application is hampered by the complexity of the method.

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The observations carried out in the current year by I. P. Zamuriy and P. P. Samoylov showed that where combination methods of investigating cattle for brucellosis were introduced, using the complement fixation reaction, the farms were almost completely freed of brucellosis. However, where the laboratory was not familiar with the complement fixation reaction or did not know how to use it, the fight against brucellosis was less successful.

Z. N. Kostrulina showed that in 20 out of 30 experimental sheep the complement fixation reaction did not disappear until 349 days after the infection with brucellosis had expired, while in the drop-plate agglutination method all sheep used in the experiment, except one, started giving negative indications after 220 days, and with the test-tube method 109 days after the infection with brucellosis.

Allergy tests to abortin, brucellizate, and brucellohydrolyzate are suitable, but they give positive indications even in those cases where the infection has already diminished or entirely died out. Brucellohydrolyzate does not induce the formation of agglutinins in inoculated animals. In this lies its advantage over the other allergens.

By combining the serum reaction with the allergy test, it is possible to eliminate infections except protracted ones in large herds, where the majority of the animals of the herd have recovered, but where there are still some cases of lingering infection. In such cases protective inoculations to provide immunity for the young are indispensable. In individual cases and in cases of fresh infection, accompanied by mass miscarriages, a herd cannot be freed of brucellosis merely by detection and isolation of animals which react positively.

If the protective inoculations are to achieve their aim, such methods should be employed which will not cause serious reactions in the animals being inoculated, which will not endanger the neighboring animals, and which will give the animals being inoculated the quickest and most lasting resistance to brucellosis.

By accumulation from year to year of such brucellosis-resistant cattle at farms affected by disease and by replacement, it is possible, within 3-6 years to restore to health an old infected herd in which mass miscarriages had occurred. In less serious cases this can be accomplished in a shorter period. Farms with fresh infection and with a small percentage of animals affected by brucellosis are freed from brucellosis considerably faster by detection and isolation of the infected animals.

At present, the so-called Strain 19 of the Type *Brucella abortus bovis* with reduced virulence is the most suitable for mass inoculation of calves.

It is recommended that protective inoculation of the young with Strain 19 be carried out when they are from 4 months to one year old. Some researchers consider it possible to inoculate calves up to the time they are mated. Only during the second half of pregnancy is inoculation categorically contraindicated.

Professor Yuskovets cites his findings to the effect that inoculation of pregnant cows is not accompanied by miscarriage.

Vaccination with Strain 19 is accompanied by the formation of agglutinins in the vaccinated animals. They are retained in animals up to one year old for about 4 months, and in grown animals for years. The vaccinated animals are not dangerous to nonvaccinated ones. They acquire immunity within 3 months after the vaccination, and remain immune for up to 19 months. Nonsterile immunity sets in considerably earlier. Prof. Yuskovets believes that vaccination with Strain 19 provides immunity within 6 to 8 weeks. It is recommended that repeated vaccinations of animals be carried out after expiration of one year, although individual animals vaccinated, according to foreign data, are supposed to acquire immunity which is retained through the third, fourth, and even fifth pregnancy.

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Apparently, in these cases resistance is increased as a result of natural infection. Soviet authors do not share the opinion that immunity persists through the third or fourth pregnancy.

Vaccinations with Strain 19 in the USSR have been carried out by the Kirov Scientific Research Institute of Epidemiology and Hygiene since 1944. There has been prepared a dry vaccine which has the advantage over the liquid bouillon of retaining its properties for a longer time (dry brucellae do not die as quickly as those in a liquid nutrient medium). However, even the drying by itself leads to the death of a considerable number of brucellae. The injection is made subcutaneously on the dewlap or the neck. The dose is 5 to 6 ml at a concentration of 20 mg of brucellae per 1 ml. It is either limited to one injection, or two vaccinations with an interval of 14 days between them are carried out. Some people consider it more effective to carry out extracutaneous vaccination with a dose of 0.2 to 1.0 ml.

The intracutaneous vaccination saves vaccine and does not lower immunogenic power of the inoculation. French authors (J. Verge) believe that immunity in vaccination with Strain 19 has the character of premonition and that its duration is determined by prolonged retention in the organism of the animal of the brucellae introduced.

G. M. Bosh'yan maintains that immunity in any infection is nonsterile. He considers as a direct proof of this the fact that original cultures are isolated from dead vaccine, serum, antigens and allergens, toxins, and antitoxins.

Up to the publication of Bosh'yan's opinion, the predominating opinion of Soviet brucellosis researchers was that protected animals acquire nonsterile immunity which later changes to sterile immunity. This view is being held even today.

The testing of the antibrucellosis semiliquid formol vaccine of Murovtsev and Trokin was begun in 1942. Starting in 1944, the vaccine has been used in large-scale tests at kolkhozes and sovkhoses in a number of republics and oblasts. Data on the results of the use of the vaccine are contradictory. Thus, it was noted, for instance, that on some farms, despite the use of the vaccine through from 2 to 5 years, miscarriages did not cease, while on others they appeared after vaccination. It was noted that after cows had been inoculated with the vaccine, their milk productivity dropped, and that up to 5 percent of the inoculated animals reacted to the agglutination test for 14 to 16 months in brucellosis tests. Such long retention of the reaction in some of the animals does not permit distinguishing whether the reaction is due to the vaccination or to natural brucellosis.

According to the conclusion of the commission which did this work, no positive results were obtained from vaccination in Vladimir Oblast.

According to the conclusion of the chief of the Veterinary Administration of the Ministry of State Farms USSR, the vaccine is harmless. The use of the vaccine on farms which have long been infected contributes to the decrease in the number of miscarriages in the first year after vaccination. On other sovkhoses, the miscarriages continue. On farms recently infected, formol vaccine inoculations did not prevent miscarriages in cows for a year after inoculation. The use of formol vaccine for therapeutic purposes yielded no results.

The chief of the Veterinary Administration of the Ministry of State Farms Ukrainian SSR reported: "No definite conclusions on the results of the use of formol vaccine can be made. Only a definite lowering of the number of miscarriages and of other clinical manifestations of brucellosis can be noted."

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In 1951, the Ministry of Agriculture USSR proposed continued use of the semiliquid formol antibrucellosis vaccine on farms of individual oblasts.

It is evident from the above data that the vaccine made from Strain 19 yielded definitely positive results, while the results obtained with the Murom-tsev-Tronin vaccine were doubtful.

There are two more vaccines: Strain 61 of the Type Brucella suis from the brucellosis laboratory of the All-Union Institute of Experimental Veterinary Medicine, and the crystal violet vaccine of Zhovaniuk from the Ukrainian Institute of Experimental Veterinary Medicine. These vaccines are in the testing stage although preliminary experiments give basis for assuming that they are of practical use.

The vaccines proposed by P. A. Vershilov and A. Kh. Kotlyarova have been tested on guinea pigs but have not been tested on cattle or other livestock.

The saponin vaccine of Professor M. I. Ivanov, tested by Skornyakov and used mainly on sheep, has not yet been approved.

We dispose of comprehensive means for the fight against brucellosis of cattle. With the aid of these means, the liquidation of the infection is entirely possible. Existing methods of diagnosing brucellosis in cases of fresh initial infection and even in cases of older infection, with 15 percent of the animals still showing a positive reaction, guarantee the recognition and removal of all carriers and transmitters of the infection by the expedient of combining these methods. A limitation must be made here. An experiment has shown that on some farms cleared of brucellosis, and even on those where all livestock had been exterminated and replaced by a new, healthy herd, brucellosis soon reappeared. Measures had been taken to protect the healthy herd, new isolated barns for the calves had been built, and these calves were cared for by separate attendants and given safe milk. Despite this, in many cases, animals came down with brucellosis upon reaching sexual maturity. In connection with this, the teaching of Academician Ye. N. Pavlovskiy on natural foci of brucellosis must be considered. The causative factor circulates from one animal to another through a transmitter or through contact. A natural focus is formed, consisting of a complex system of interrelations between causative factors, transmitters and carriers of the causative factor of the disease, and the external medium surrounding them. A study of each of these components is indispensable.

In addition to livestock, dogs, cats, marmots, hamsters, field mice, muskrats, reptiles, amphibia, and wild animals related to sheep, goats, swine, dogs, and cats are susceptible to brucellosis. The brucellae, discharged by the animals, keep infiltrating the ground, the wells, and the pastures. The conditions are established for infection of animals and birds susceptible to brucellosis. Its spread is even possible through blood-sucking arthropodes (ixodes ticks) which have been proved to be carriers of the causative factor of brucellosis (Samsonov). In the fight against brucellosis up to now, natural foci of infection have not been considered although this obviously an essential part of the fight. As long as the theory of natural foci of brucellosis corresponds to the actual situation -- which has been confirmed by new outbreaks of the infection in herds which had been freed from the disease -- then the vaccination of the animals and providing them with permanent immunity becomes an obligatory measure for all farms where natural foci of brucellosis exist. The study of this question is a matter of great importance.

The most simple and generally recognized combined diagnostic method for cattle is the agglutination reaction plus the allergy test. The complement fixation reaction may be used only in doubtful cases or where it is easily carried out. Serological investigations, carried out at 3-week intervals, suffice for the successful detection of brucellosis carriers or secretors. Elimination of the animals which are brucellosis carriers is the basic task in liquidating the infection.

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Eradication of sources of infection such as polluted litter, manure, liquid manure, water polluted with waste, miscarriages, afterbirths, exudates of genital ducts, should be carried out by special disinfection methods. Milk from cows suffering from brucellosis and dairy products which may be infected (fresh cheese made from goat or sheep milk) are required to be pasteurized. On farms with protracted infection and a high percentage of animals giving a positive reaction (above 15 percent) or on farms where there are few serological but many allergy reactions, the fight against brucellosis cannot be limited only to diagnostic control and elimination of the positively reacting animals. There protective vaccination of the young from 4 months to one year of age or up to 15 months of age with vaccine of Strain 19 is obligatory.

Some advise that calves be vaccinated with Strain 19 after a preliminary negative check for brucellosis and furthermore recommend carrying out this vaccination even at the time of the first mating and repeating it every year before impregnation.

According to investigations of the brucellosis laboratory of the All-Union Institute of Experimental Veterinary Medicine, the higher the age of the animals to be vaccinated, the greater the danger of miscarriages. Their percentage increases with the age of the animal. Agglutinins are retained for a long time in older animals. Therefore, the age from 4 to 10 months is the most suitable for vaccination with Strain 19.

Soviet veterinary investigators have determined the duration of immunity conferred by Strain 19 at 19 months, and figure 3 months for the onset of immunity in all vaccinations. Nonsterile immunity sets in much earlier. Consequently, the animals inoculated in a brucellosis-infected herd should be kept isolated for 3 or at the least for 2 months.

Vaccinations with dry vaccine are preferable because of its longer retention. The intradermal method of vaccination represents a saving in vaccine, since the dose is one fifth of that used in subcutaneous vaccination.

Two methods of fighting against brucellosis are known. The first is based on the detection and elimination of the sources of infection, the second on providing the animals with artificial immunity, but not excluding the removal of those animals which are known to be carriers of the infection. Both methods are used and lead to successful results. In case of a limited number of animals with positive reaction, the first method quickly rids the herd of infection. The second method, which is more drawn-out, requires yearly inoculation for 3, 5, or 6 years. This method has another drawback. It gives rise to the formation of agglutinins in vaccinated animals and simulates natural infection. Some vaccinated animals do not become properly resistant and have miscarriages. Nevertheless, this method limits the spread of brucellosis and generally rids badly infected herds of the disease. We have examples of liquidation of brucellosis within 2 or 3 years after vaccination with Strain 19.

So far, spontaneous recovery of cattle herds has not been considered as a means in the fight against brucellosis from the practical standpoint. To be sure, a high percentage of the animals do recover by themselves, but the role of this factor in the fight against brucellosis is slight, since not all animals recover and this process of recovery varies in length. It is essential that continuous serological investigations of the animals be made and that the animals which show a protracted serological reaction be removed from the herd. The allergy reaction by itself is not suitable for evaluating spontaneous recovery because of its protracted retention by the majority of formerly infected animals. If the reaction is lost together with the serological reactions, or if it is retained after the latter have faded out, it is an indication that spontaneous recovery has set in.

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The question of spontaneous recovery of sheep from brucellosis has not been sufficiently illuminated until recently. Kh. S. Kotlyarova and P. A. Vershilova found early loss of brucellae capable of forming cultures upon seeding, 3 to 4 months after infection in some cases, and in 13 to 41 months in all investigated groups. At the same time, these authors found prolonged discharge of brucellae in the urine. Ye. S. Orlov found prolonged discharge of brucellae with the milk after puncture of the udders. Admitting the certainty of spontaneous recovery of sheep from brucellosis with prolonged retention of the causative factor in only one fifth of animals within 2 years after infection, Kotlyarova and Vershilova speak of the possibility of prolonged local infection (mastitis, orchitis). I. R. Zamuriy and D. K. Bessonov established the spontaneous recovery of sheep in brucellosis isolators from the loss of the serological reactions.

Z. N. Kostrulina and P. P. Samoylov carried out a 19-month observation on 30 experimentally infected and naturally infected sheep. In the course of 349 days, 20 out of 30 sheep lost the complement fixation reaction (Wright's reaction was lost considerably earlier). The test-tube method of the agglutination reaction gave negative indications in 28 sheep as early as 109 days after infection. The spot-plate or drop method of agglutination with serum gave positive indications up to 220 days in all the sheep under examination, with the exception of one. In 23 sheep, the allergy reaction disappeared after 11 months. A check by autopsy on several sheep which had lost all diagnostic reactions, and also on those which had retained the allergy reaction but lost the agglutination and complement fixation reactions, gave a negative result in attempts to grow cultures and to infect guinea pigs.

Thus, spontaneous recovery of experimentally infected sheep of the Romanov breed in the course of 11 months can be considered as proved. Spontaneous recovery of sheep afflicted with natural brucellosis, which were isolated, was also proved. A test by the blood-drop method of agglutination of sheep infected with brucellosis which had yielded a positive result according to Il'in, gave a negative result according to Z. N. Kostrulina. The blood-drop method has considerable advantages in practical work and gives a simple and easy diagnosis of brucellosis in infected herds on the spot. Therefore, further perfecting of this method and its introduction into practical work, particularly for the diagnosis of brucellosis in sheep, will be of great importance.

The fight against brucellosis of sheep and goats offers the same possibilities as the fight against brucellosis of beef cattle. removal of the sources of infection by detection of sick animals through diagnostic methods, eradication of the causative factors of infection in the environment, and increasing the resistance of the animals by artificial immunization (vaccination). Until recently, the allergy method with the aid of brucellizate as proposed by the field team of the All-Union Institute of Experimental Veterinary Medicine served as the only diagnostic method for the recognition of dangerous brucellosis infection of sheep and goats, according to the instructions of the Veterinary Administration of the Ministry of Agriculture USSR. Recently, it has been discovered that brucellosis of sheep cannot be eliminated with the aid of this method alone. Nonspecific reactions to brucellizate were proved, occasional cases of discharge of brucellae by sheep which had not reacted to brucellizate were established, and data were compiled which indicated the futility of attempts to liquidate brucellosis with the use of the allergy method alone.

It is, therefore, necessary to resort to supplementary serological tests, i.e., the agglutination reaction and the complement fixation reaction, to attain more effective results. But even brucellhydrolyzate, although it has advantages over brucellizate, cannot detect all brucellosis-infected animals without additional serological diagnostic reactions, and consequently, permit to rid farms of the infection. Combined application of serological and allergy tests gives better results. However, the test-tube agglutination method, as already pointed out, does

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not give satisfactory results in brucellosis of sheep, while the drop method is acceptable and practicable. The complement fixation reaction, in the practice of a number of veterinary researchers, has yielded better results than the agglutination reaction, although the complexity of applying it on a large scale hampers its application.

Brucellosis takes a different course at different farms. A more favorable or less favorable course of the infection, a large or small number of miscarrying animals, increasing number of cases of sterility or stillbirths at one farm and a decreasing number at another farm are observed. It is known that the moment of infection with brucellosis influences the number of miscarriages. Infection up to the time of mating generally does not result in a miscarriage, while infection during pregnancy leads to a diseased condition of the pregnant uterus and to miscarriage.

The outcome of brucellosis is also influenced by the dose of the causative agent, as well as by the conditions under which the animals are kept, the condition of feeding, of the treatment, the presence of vitamins and phosphorus-calcium salts in the feed, climatic influences, chilling, excessive heat, overwork, and other factors. Elimination of these outside influences and establishing of favorable conditions for the animals should be of help in stamping out the infection.

Of the veterinary measures for the liquidation of brucellosis in cattle, in cases of initial infection and in cases of a small percentage of affected animals (less than 15 percent), the agglutination reaction in combination with the allergy and complement fixation reactions (the latter particularly for deciding doubtful cases) guarantees quick ridding of the farm from the disease. If the percentage of animals affected by brucellosis is higher (above 15 to 20 percent), the sero-allergic diagnosis must be accompanied by vaccination of the young from 12 to 15 months of age and sometimes by vaccination of the entire herd. For keeping the farm free from disease afterwards, careful disinfection must be carried out.

In brucellosis of sheep, removal of the infection is accomplished by the combined sero-allergic method (agglutination reaction plate test with allergy test) and by the complement fixation reaction, wherever possible. Spontaneous recovery of the animals can also be used for liquidating brucellosis of sheep. In flocks diseased with brucellosis, with a high spread of the infection, one may organize an isolated flock of adult sheep and periodically examine their sero-allergic reaction. Clinically sick sheep and those reacting to the agglutination test and the complement fixation reaction are slaughtered. Loss of the serological and allergy reactions indicates the spontaneous recovery of the animals. A negative serological reaction with retention of a positive allergy test also indicates spontaneous recovery. However, it must not be forgotten that infection may persist in individual cases, even though all diagnostic tests turn out negative. The presence of only one allergic positive test even in rare cases does not exclude the possibility of infection. The criterion for recognizing the freedom of a herd from the infection is the prolonged absence of miscarriages and serological health.

P. F. Samoylov carried out autopsies and bacteriological investigations on 20 slaughtered sheep which had given negative serological and positive or doubtful allergy reactions. Despite the investigation of the lymphatic nodes, the bone marrow, and the parenchymatous organs, he could not isolate a brucella culture. In individual sheep which had retained the serological reaction, on the other hand, brucella cultures were isolated. A total of 47 sheep was investigated by autopsy and bacteriological tests. Brucella cultures were isolated from four sheep. Three sheep which had lost both the serological and the allergy reaction were found to be free of brucellosis.

Analogous results were also obtained on experimental sheep.

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2 For the quickest liquidation of brucellosis in agricultural animals the following steps are essential:

a. Perfection and introduction into practice of the blood-drop method of agglutination.

b. Providing oblast and rayon laboratories (scientific research veterinary experimental stations) with components for the complement fixation reaction (with dry complement, hemolytic serum, and antigen) and for widening the application of this reaction.

c. Acceleration of the development and introduction into practice of methods of specific prophylaxis and treatment of brucellosis in animals.

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